Communications to the Editor

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TANGSHENOSIDES I AND II FROM CHUAN-DANGSHEN, THE ROOT OF *CODONOPSIS TANGSHEN* OLIV.

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From the roots of Codonopsis tangshen (Chuan-dangshen) cultivated in Sichuan, China, two new phenylpropanoid glucosides named tangshenosides I and II were isolated together with syringin. the structures of both compounds including the absolute configurations were elucidated by physical and chemical procedures.

KEYWORDS —— Codonopsis tangshen; Dangshen; Campanulaceae; Chinese folk medicine; tangshenoside I; tangshenoside II; syringin; phenylpropanoid glycoside;

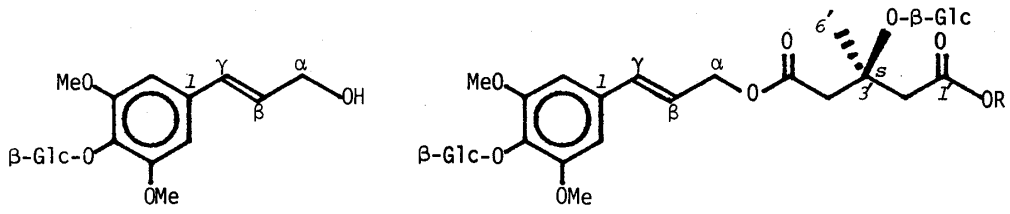
Dangshen (党参), Codonopsis Radix, is a very common traditional crude drug in China and described as the roots of Codonopsis pilosula (Franch.) Nannf. (Campanulaceae), (Lu-Dangshen,潞党参, Xi-Dangshen,西党参, Dong-Dangshen,東党参) or C. tangshen Oliv. (Chuan-Danshen,川党参).[1] It is also stated that the roots of a variety of other Codonopsis spp. are sometimes used as Dangshen.[1] Several studies of the chemical constituents of this crude drug have appeared in the literature.[1,2] Recently, we have reported the isolation of (Z)-3-hexenyl and (E)-2-hexenyl β-D-glucosides from Dangshen purchased in Chendu, China[3] and Wang et al. identified small amounts of atractylenolides, n-hexyl-β-D-glucoside, syringin, syringaldehyde, vanillic acid, 2-furancarboxylate, nicotinic acid and 5-hydroxy-2-pyridinemethanol in the roots of C. pilosula.[4] However, the amounts of these compounds are extremely low and no characteristic major constituents useful for the chemical identification and evaluation of this drug, have been isolated. This difficulty is mainly due to the high content of water-soluble carbohydrates; more than 90% of the methanolic extract of this drug consist of sucrose and other water-soluble saccharides. The present communication deals with the isolation and structural elucidation of two new glucosides from the roots of C. tangshen cultivated at Wan Xian, Sichuan, China.

An aqueous solution of the methanolic extract (1.8 kg) of the dried roots (5.5 kg) was chromatographed on highly porous polymer (Diaion HP-20) to remove a large amount of sucrose and other saccharides (total about 1.6 kg) by eluting with water. A fraction (33 g, yield: 0.6%) eluted with 30% methanol was chromatographed on

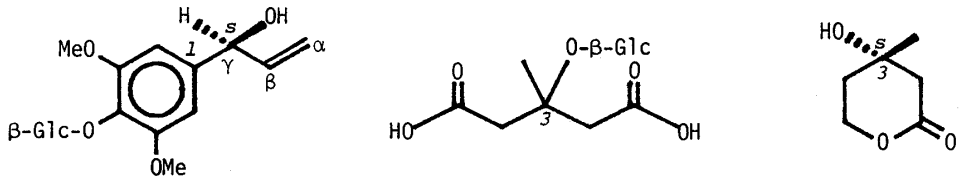
silica gel [solvent: $CHCl_3$-MeOH-H_2O (10:5:1-6:4:1) graduent]. It was separated into ten fractions, fr. 1-10. The less polar fraction, fr. 1 (1.1 g) was chromatographed twice on silica gel [solvent: $CHCl_3$-MeOH-H_2O (60:15:1) and then (30:10:1)], then on a reverse phase column [LiChroprep RP-8 (solvent: 30% MeOH)].It was finally subjected to preparative high performance liquid chromatography (HPLC) on a reverse phase column [TSKgel ODS-120T (solvent: 12% CH_3CN) to give two compounds, **1** (96 mg) and **2** (29 mg). The more polar fraction, fr. 7 (6.8 g) was chromatographed on Sephadex LH-20 (solvent: 50% MeOH) and then twice on LiChroprep RP-8 (solvent: 20% MeOH and then 18% MeOH). It was finally purified by preparative HPLC on TSKgel ODS-120T (solvent: 10% CH_3CN) to give compound **3** (1.7 g).

Compound **1** was identified as syringin.[5] A new compound **2**, named tangshenoside II, an isomer of **1** (FD-MS $[M+Na]^+$ m/z 395), a white powder, $[\alpha]_D^{19}$ -29.0° (c=1.37, EtOH) showed UV absorption, λ_{max}^{MeOH}nm(logε) 230 (3.7) and 268 (2.9). On hydrolysis with β-glucosidase (emulsin), **2** yielded D-glucose and the ^{13}C-nuclear magnetic resonance (NMR) spectrum of **1** (Table) indicated the presence of a β-glucopyranoside unit. The ^{1}H-NMR spectrum of **2** (in CD_3OD) exhibited signals due to the aglycone moiety; δ 3.84 (6H s, $-OCH_3$ x 2), 6.71 (2H s, two equivalent aromatic protons) and a set of signals due to phenyl-CH(OH)-CH=CH_2, 5.07 (1H d, J=6.1Hz, carbinyl proton), 5.99 (1H ddd, J=16.8, 10.1 and 6.1Hz, olefinic proton), 5.13 (1H dd, J=10.1 and 2.8Hz vinylic proton (Z)) and 5.31 (1H dd, J=16.8 and 2.8Hz, vinylic proton (E)). As a result the structure of **2** was assigned as shown in the Chart. The location of the glucoside linkage on the phenolic hydroxy group is based on the negative $FeCl_3$ test. The carbon signals due to the aglycone moiety of **2** were assigned by the C-H COSY procedure (Table), supporting this formulation. The chirality of the γ-carbon of **2** was assigned to be S-configuration as follows: **2** (3mg) was hydrolyzed with emulsin and the products were separated by chromatography on Diaion HP-20. After removing glucose by elution with water, the column was eluted with methanol to give the aglycone which was subjected to the microscale determination of the chirality of a secondary hydroxyl group (Horeau method modified by Brooks and Gilbert).[6]

Another new compound,**3**, named tangshenoside I, a white powder, $[\alpha]_D^{17}$ -22.0° (c=0.55, H_2O), FD-MS $[M+Na]^+$ m/z 701, UV $\lambda_{max}^{H_2O}$nm (logε) 226(4.3) and 266(4.4), gave D-glucose on hydrolysis with emulsin. On treatment with diazomethane, **3** afforded a methyl ester (**4**), a white powder, $[\alpha]_D^{19}$ -20.1° (c=0.79, H_2O), UV $\lambda_{max}^{H_2O}$nm (logε) 227(4.1) and 266(4.2). The ^{13}C-NMR spectra of **3** and **4** (Table) as well as the anomeric proton signals [**3**: δ 4.58 (1H d, J=7.3) and 4.83 (1H d, J=7.6Hz) in D_2O, **4**: δ 5.19 (1H d, J=7.6Hz) and 5.83 (1H d, J=7.0Hz) in C_5D_5N], indicated the presence of two β-glucopyranoside units in **3**. On alkaline hydrolysis, **3** yielded syringin (**1**) and an acidic glucoside (**5**), colorless syrup, $[\alpha]_D^{18}$ -17.9° (c=2.11, H_2O). On hydrolysis with emulsin followed by methylation with diazomethane, **5** afforded dimethyl 3-hydroxy-3-methylglutarate which was identified by comparison of gas liquid chromatogram (GLC) with an authentic sample. These results led to the formulation of **3** as shown in the Chart. The ^{13}C-NMR (Table) and the ^{1}H-NMR spectra of **3** and **4** are consistent with this structure; ^{1}H-NMR spectra: **3** in D_2O δ 1.41 (3H s), 2.47 (1H d, J=15.1Hz), 2.61 (1H d, J=15.1Hz), 2.82 (2H s), 3.75 (6H s), 4.65 (2H d, J=6.1Hz), 6.17 (1H dt, J=6.1 and 16.1Hz), 6.52 (1H d, J=16.1Hz) and 6.66 (2H s), **4** in C_5D_5N δ 1.81 (3H s), 3.23 (1H d, J=15.6Hz), 3.25 (1H d, J=15.6Hz), 3.31 (1H d, J=15.6Hz), 3.42 (1H d, J=15.6Hz), 3.58 (3H s), 3.79 (6H s), 4.87 (2H d, J=6.4Hz),



1 3 : R=H 4 : R=Me



2 5 6

Chart

Table. ^{13}C-Chemical Shifts

	1[a]	2[b]	3[c]	4[a]	5[c]	HMG[c),d]
C - 1	134.0	140.5	134.2[e]	134.1		
2,6	105.2	104.3	105.1	105.7		
3,5	153.9	153.2	153.3	153.9		
4	135.7	134.2	134.4[e]	135.7		
-O$\underline{C}$H$_3$	56.6	56.0	57.0	56.6		
α	62.8[e]	114.0	66.3	65.2		
β	129.4	140.9	124.3	123.2		
γ	131.1	75.0	134.4	132.8		
1'			173.3	171.6[e]	177.4	175.8
2'			44.3	44.0	46.4	46.0
3'			78.2	76.5	78.2	70.7
4'			47.4	44.0	46.4	46.0
5'			176.7	171.1[e]	177.4	175.8
6'			24.8	25.0	24.9	27.2
-COO$\underline{C}$H$_3$				51.6		
G - 1	104.9	104.3	103.8	104.8		
2	76.1	74.7	74.5[f]	76.2		
3	78.7[f]	77.3[e]	77.0[g]	78.8[f]		
4	71.6	70.3	70.3	71.7		
5	78.3[f]	76.8[e]	76.6[g]	78.4[f]		
6	62.6[e]	61.5	61.5[h]	62.6[g]		
G'- 1			97.2	98.7	97.2	
2			74.0[f]	75.1	73.9	
3			76.6[g]	78.6[f]	76.5	
4			70.0	71.7	70.4	
5			76.5[g]	78.4[f]	76.5	
6			61.2[h]	62.8[g]	61.6	

a) Measured in C_5D_5N.
b) Measured in CD_3OD.
c) Measured in D_2O.
d) HMG: 3-hydroxy-3-methylglutaric acid(authentic sample).
e,f,g,h) May be exchanged in compound.

6.38 (1H dt, J=6.4 and 15.9Hz), 6.69 (1H d, J=15.9) and 6.86 (2H s). The chirality of the 3-O-β-D-glucopyranosyl-3-methylglutarate moiety of **3** was established as follows: **3** was hydrolyzed with emulsin and the resulting aglycone was subjected to diborane reduction followed by alkaline hydrolysis to give mevalonolactone (**6**). The microscale determination of the chirality of mevalonolactone has been conducted by conversion into the trimethylsilyl ether of 3(R or S)-1-[(R)-phenylethyl]-mevalonamide and subsequent GLC analysis.[7,8] By this procedure, **6** was identified as 3(S)-mevalonolactone. Consequently, the chirality of the 3' position of **3** was assigned as the S configuration.

Tangshenoside I (**3**) was also isolated from the commercial Dangshen purchased in Sichuan, China. Because the amount of **3** is significantly more than any the other compounds that have ever been isolated from this crude drug, this glycoside is valuable as a marker substance for the chemical identification of Dangshen.

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